PERISTALTIC PUMP DESIGN AND EVALUATION FOR BIOREACTOR FEEDING STRATEGIES

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ABSTRACT

Increasing the efficiency of semi-continuous bioprocesses requires precise control of liquid flow, which is critical for optimal nutrient delivery or waste removal in bioreactors. To address this need, the construction and performance evaluation of a peristaltic pump featuring a Proportional-Integral-Derivative (PID) controller for bioreactor feeding strategies was conducted. The low-cost, high precision pump is constructed using 3D-printed components and is optimized to provide precise control of fluid flow. The use of a PID controller allows for dynamic adjustment of flow rates to meet specific feeding requirements with high accuracy. Experimental tests demonstrate the pump's effectiveness in managing fluid delivery across different feeding profiles - constant, linear, and exponential. The cultivation of *Bacillus subtilis* in a stirred-tank bioreactor indicates significant improvements in biomass production during the feeding period, showing the critical role of precise volume management in optimizing fed-batch bioprocesses.

Keywords: Peristaltic pump. 3D printing. PID controller. *Bacillus subtilis* cultivation. Semi-continuous mode.

1 INTRODUCTION

A peristaltic pump employs rollers on a rotor to compress and release tubing, propelling fluid from inlet to outlet in a process reminiscent of human peristalsis. Its resilience against high back pressures and its ease of sterilization - since the fluid contacts only the tubing interior - make it an ideal choice for precise fluid control in various applications [1]. This study focuses on the construction and assessment of a peristaltic pump equipped with a PID (Proportional-Integral-Derivative) controller, a system that dynamically adjusts flow rates to match specified feed batch profiles with high accuracy [2].

2 MATERIAL & METHODS

The pump was constructed using the Anycubic® UV Tough Resin with the Anycubic® Photon Mono M5s 3D printer. The pump core consists of three main components: the motor, the rotary head, and the stationary pressure head. A NEMA17 stepper motor, regulated by an MKS SERVO42C closed-loop controller, directly drives the rotary head. This rotary head features a resin frame embedded with six metal bearings and the stationary head includes adjustable pressure settings to accommodate hoses of varying diameters. For flow control, a Proportional-Integral-Derivative (PID) controller algorithm was implemented in C++ on an ESP32-DevKitC development board. To assess the precision of the pump's flow control, the values obtained from the controller were compared with experimental volume data. To evaluate the effects of the peristaltic pump's operation, *Bacillus subtilis* was grown in a stirred-tank bioreactor (TECNAL® TEC-BIO 7.5 VI) operated on fed-batch mode with 2 L initial medium at 37°C and pH was maintained at 7. The cells grew in the batch mode for 10 h after inoculation, and then the feeding process was continued from 10 to 58 h. The fed-medium contained 400 g/L of sucrose, 10 g/L yest extract, 10 g/L peptone, 10 g/L $NaNO_3$, 4 g/L Na_2HPO_4, 4 g/L KH_2PO_4, 0,05 $FeSO_4$, 0,05 g/L $ZnSO_4$. Agitation rate was 100 – 1000 rpm to maintain the OD at 30%, with an aeration rate of 5 L/min. The oxygen transfer rate (OTR) was calculated through the gas balance using the BlueSens® BlueInOne Ferm sensor. Biomass concentration was quantified via DO 600 nm with the Thermo Scientific® GENESYS 10S spectrophotometer.

3 RESULTS & DISCUSSION

Delving into the mechanics of peristaltic pump operation reveals intricate physical relationships governing volume displacement. In the context of a full rotational cycle, the trajectory delineated by the portion of the motor compressing the hose equates to the effective circumference. This relationship defines the displacement volume (V_{rot}) calculation for a complete rotation, derived from multiplying the hose's cross-sectional area ($\pi {R_{hose}}^2$) by the path length covered during the rotation ($2\pi R_{rotor}$). The volume displacement (V_θ - in cubic meters) for a given angular change $\Delta\theta$ (in radians) scales with the angle's proportion to a full 360-degree rotation and the volume fraction occupied by the rollers [3] (V_{roller} – calculated to be about 30% in our design), as delineated in Equation 1. In stepper motor-driven peristaltic pumps, the volumetric output is inherently discretized by the steps of the motor. Microstepping enhances this arrangement by subdividing each step into finer increments, significantly improving the precision of the output. This process fine-tunes $\Delta\theta$ by adjusting the motor's step angle (θ_{step} – radians per step) and the microstepping configuration (M), enabling precise control over the pump's flow rates. Consequently, this adjustment allows for the accurate computation of the volume displaced per step (V_{step} - cubic meters per step), as shown in Equation 2. Figure 1 visually depicts the pump head, illustrating the key structural variables involved.

$$V_\theta = V_{rot} \cdot \Delta\theta/2\pi \Longrightarrow V_\theta = \pi {R_{hose}}^2 2\pi R_{rotor}(1 - \%V_{roller}) \cdot \Delta\theta/2\pi \Longrightarrow V_\theta = \pi {R_{hose}}^2 R_{rotor}(1 - \%V_{roller})\Delta\theta \quad (1)$$

$$V_{step} = V_{rot} \cdot \theta_{step}/M \Longrightarrow V_{step} = \pi {R_{hose}}^2 R_{rotor}(1 - \%V_{roller}) \cdot \theta_{step}/M \quad (2)$$

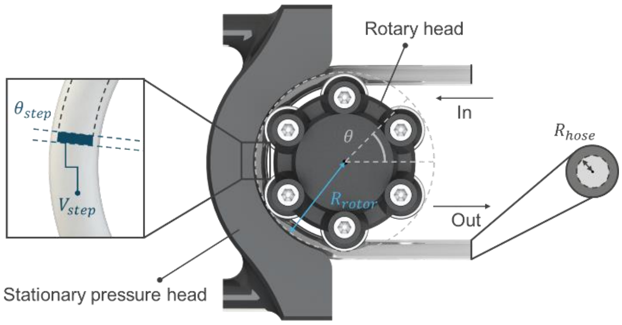

Figure 1 Demonstration of the peristaltic pump's rotor with key parameters highlighted.

The angular velocity of the motor (ω - radians per second), as expressed in Equation 3, is directly proportional to the number of steps per second (N_S) and to the conversion factor for radians and microstepping (θ_{step}/M). Consequently, the flow rate (Q - cubic meters per second) is defined by multiplying ω and V_{rot}, as delineated in Equation 4:

$$\omega = N_S \cdot \theta_{step}/M \tag{3}$$

$$Q = \pi R_{hose}{}^2 R_{rotor}(1 - \%V_{roller}) \cdot N_S\, \theta_{step}/M \Longrightarrow Q = V_{step} \cdot N_S \tag{4}$$

The relationship between volume and flow rate parallels the correlation between the cumulative number of steps (S) and N_S. This serves as a pivotal calibration factor, aligning the volumetric output per step (V_{step}) precisely with the actual flow rate (Q) or the cumulative volume output up to a given point in time (V) with S. Consequently, it becomes feasible to establish equations for various feeding profiles characterized by flow rate variations over time, including constant, linear, and exponential profiles [4]. Table 1 outlines the computation of S for different feeding strategies.

Table 1 Calculation of cumulative step outputs (s) for different feeding strategies.

Feed type	Flow rate Q (m³/s)	S (cumulative steps)
Constant	$Q = \lambda$	$S(t) = V_{step}{}^{-1} \int_0^t \lambda\, d\tau = \lambda t / V_{step}$
Linear	$Q(t) = \lambda + \phi t$	$S(t) = V_{step}{}^{-1} \int_0^t \lambda + \phi\tau\, d\tau = \phi t^2/(2V_{step}) + \lambda t/V_{step}$
Exponential	$Q(t) = \lambda e^{\phi t}$	$S(t) = V_{step}{}^{-1} \int_0^t \lambda e^{\phi\tau}\, d\tau = \lambda e^{\phi t}/(\phi V_{step})$

Through the examination of a three-minute peristaltic pump operation test, aimed at evaluating the pump's capability to handle a 10 mL volume within this timeframe, findings are showcased across three distinct flow profiles: constant, linear, and exponential. With a θ_{step} of 1.8° and a microstepping setting (M) of 16, the pump achieved a resolution of around 0.145 μL per step. The test results, depicted in Figure 2, emphasize the efficacy of the PID control mechanism in achieving precise volume management, further highlighted by the pump's ability to finely tune its output to meet specific flow requirements.

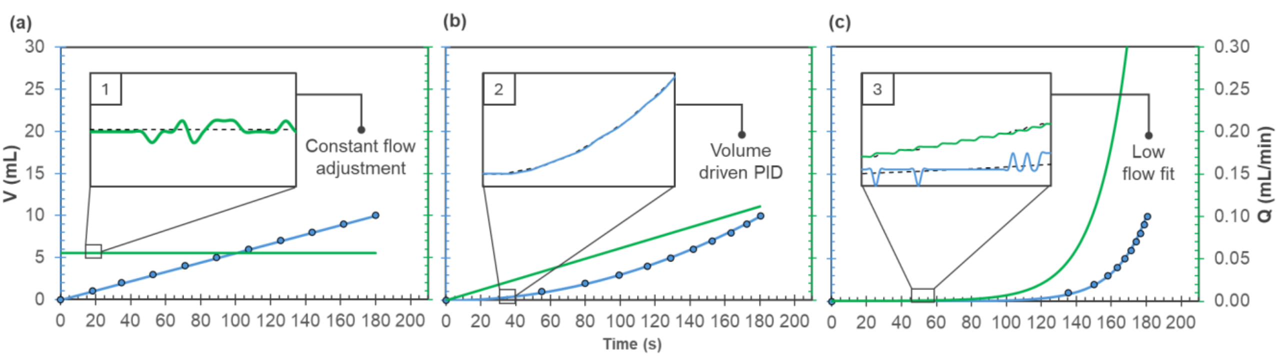

Figure 2 Volume and flow rate analysis for a 10mL runs under (a) constant, (b) linear, and (c) exponential flow profiles, highlighting the precise flow adjustments, volume-driven PID control, and the system's adaptability at low flow rates.

The essence of the PID controller's operation lies in its ability to continuously modulate the pump's flow rate to align with predetermined volume change. This is achieved by adjusting the motor's stepping frequency in real-time, a process dictated by the calculation of proportional, integral, and derivative terms based on the discrepancy between the targeted and actual step counts, as shown in Figure 3. Specifically, the PID algorithm dynamically refines the motor's speed to either ramp up or down the flow rate, ensuring that the actual volume dispensed adheres closely to the intended profile calculated by $S(t)$.

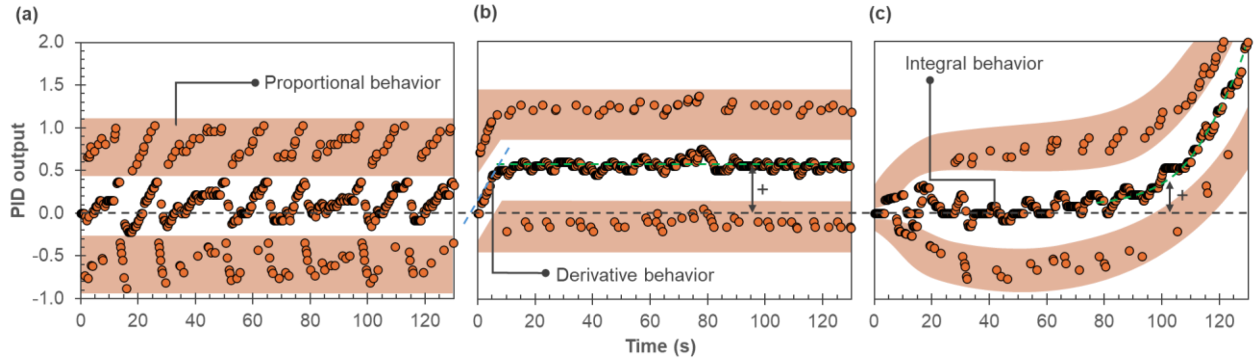

Figure 3 PID controller output across (a) constant, (b) linear, and (c) exponential flow profiles, showcasing the controller's real-time adjustments behavior to maintain the desired volume output.

The PID control algorithm demonstrates its precise effect on cultivation results. In the case presented, the exponential feed strategy was applied by modifying the exponential equation from Table 1 to depict in terms of kinetic and physical parameters of the cultivation, as shown in Equation 5. This equation tailors the nutrient supply to the bioprocess demands, where V_0 is the initial volume (2 L), C_{X_0} the initial biomass concentration (1.945 g/L), C_{SQ} the sucrose feed concentration (400 g/L), $Y_{X/S}$ the cell yield on substrate (0.372) and ϕ is adjusted by the controller to match the total volume requirements over the period of time (t). The relationship between these variables is crucial to sustaining an optimal growth environment and is described as follows [4].

$$Q(t) = \lambda e^{\phi t} = [V_0 C_{X_0} \phi / (C_{SQ} Y_{X/S})] e^{\phi t} \quad (5)$$

Figure 4 depicts the growth of *B. subtilis* in a batch culture for the initial 10 hours and the transition to the exponential feeding regime thereafter. Notably, after the onset of the feed, the observed twelvefold increase in biomass and the pronounced elevation in oxygen uptake rate (OUR) signify a marked escalation in metabolic activity, even as the volume only increased by 2.5 times. Furthermore, the graph demonstrates the precise nutrient delivery achieved by the peristaltic pump, as evidenced by the volume profile throughout the cultivation period.

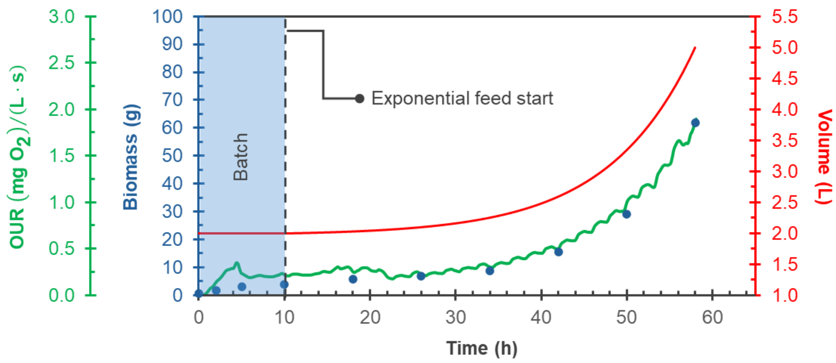

Figure 4 Impact of PID-controlled exponential feeding on *B. subtilis* growth.

4 CONCLUSION

By translating flow requirements into discrete step increments compatible with the stepper motor's operational paradigm, the controller dynamically adjusts the motor's speed based on real-time feedback, ensuring the flow remains consistent. This method demonstrates the pump's ability to accurately meet complex flow profiles with precision, showcasing its suitability for use in fed-batch operational modes.

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